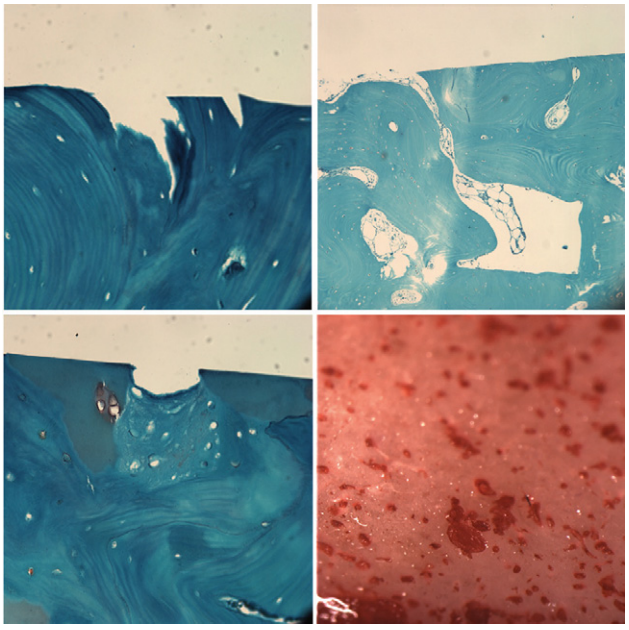

staining positive for mucopolysaccharides, type II collagen, and Lubricin. The depressions or pits were due to three conditions: aggregate erosion, vascular rupture, and dead bone fragmentation.
Conclusions: The cartilaginous aggregates have potential for proliferation contributing to cartilage repair. The multiple small pits could be the home for various cell therapies; i.e. synovial cells, stem cells, or therapeutics.

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SUSCEPTIBILITY OF CARTILAGE *IN VIVO* VERSUS CARTILAGE EXPLANTS *IN VITRO*

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Purpose: Joint bleeds lead to joint destruction. Knowledge about the mechanism of this blood-induced arthropathy has originated from both *in vitro* and *in vivo* studies. Our group has shown that *in vitro* exposure of cartilage to 50% v/v blood for 4 days leads to severe (-98%) and long-lasting (-78% after 16 days) inhibition in cartilage matrix synthesis. Also after an experimentally *in vivo* induced haemorrhage in the dog knee joint, direct harmful effects were observed, including inhibition of the cartilage matrix synthesis (-22%). But while in the *in vitro* experiments this inhibition was long lasting, in the *in vivo* experiments, effects were less outspoken and long-lasting. One of the differences between the *in vitro* and the *in vivo* situation is that in the *in vivo* situation, the cartilage is exposed to blood at the articular surface only, whereas in the *in vitro* explant culture system the cartilage is exposed to 5 additional cutting edges. Whether this difference in exposure of cartilage to blood can explain the difference between the *in vitro* and *in vivo* studies on blood-induced cartilage damage was subject of this study.
Methods: Human full thickness articular cartilage tissue was exposed to 50% v/v blood for 4 days either to all sides in an explant culture system, or in a culture system enabling isolated articular exposure (for this purpose a specific culture device was developed and validated). Subsequently the cartilage was cultured for an additional 12 days without blood to exclude the direct reversible effect. After these 16 days, cartilage proteoglycan synthesis rate and - content were determined.
Results: Exposure of cartilage to blood at all sides, both articular surface and cutting edges, led to a decrease in proteoglycan synthesis rate of -92% and a decrease in proteoglycan content of -19%. These effects were less outspoken when the cartilage was exposed to only the articular surface: -52% and -10% for proteoglycan synthesis rate and - content respectively.
Conclusions: *In vitro* exposure of cartilage to blood at the articular surface alone leads to less severe effects on the proteoglycan synthesis rate and - content than when cartilage explants are exposed at all sides. This is probably part of the explanation why blood-induced cartilage damage after an experimentally induced haemarthros *in vivo* is less severe compared to the *in vitro* effects of blood on cartilage. Irrespectively, blood has devastating effects on articular cartilage, and in this respect it is important to prevent (traumatic) joint haemorrhages and if they occur, to treat them properly. Additionally this study demonstrates that results of cartilage tissue explant cultures, exposed at all sides to culture medium and additions should be interpreted with caution.

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ACTIVATION OF VOLUME-SENSITIVE CHLORIDE CURRENT BY DOXORUBICIN IN ISOLATED RABBIT ARTICULAR CHONDROCYTES

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Purpose: Chondrocyte apoptosis contributes to the disruption of cartilage integrity in osteoarthritis (OA). Recently, it has been suggested that activation of volume-sensitive Cl- current (ICl,vol) mediates cell shrinkage triggering apoptosis (apoptotic volume decrease: AVD) in several cell types. The present study was designed to investigate the effects of a potent apoptosis-inducer, doxorubicin, on ICl,vol in rabbit articular chondrocytes using whole-cell patch-clamp technique.
Methods: Rabbit cartilages were collected from bilateral knee, hip and glenohumeral joints of male animals weighing 2.0 to 3.0 kg. The cartilage was dissected into slices and cultured in DMEM for 1-3 days. On the day of experiments, chondrocytes were isolated by enzymatic digestion. Whole-cell membrane current was recorded under conditions where Na^+, K^+ and Ca^{2+} currents were minimized. Osmolality of bath solution was adjusted with mannitol. Real-time change in cell size was monitored using a CCD digital camera and the cross-sectional area of cell image was measured.
Results: Exposure of isolated chondrocytes to doxorubicin (1 μM) resulted in a gradual loss of cell size (approximately 7% decrease in the cross-sectional area over 30 min), which was significantly attenuated in the presence of a specific ICl,vol blocker 4-(2-butyl-6,7-dichloro-2-cyclopentylindan-1-on-5-yl)oxybutyric acid (DCPIB, 10 μM). On the other hand, whole-cell patch-clamp recording revealed an obvious increase in the membrane Cl- conductance by doxorubicin without any appreciable change in cell size. The doxorubicin-evoked Cl- current exhibited many properties characteristic of ICl,vol phenotype, including outward rectification, prominent inactivation at large positive potential ($>$+50 mV), inhibition by hyperosmotic cell shrinkage, and sensitivity to DCPIB.
Conclusions: The present results suggest that doxorubicin enhances the Cl- efflux via activation of volume-sensitive Cl- channel in rabbit articular chondrocytes, which may be involved in doxorubicin-induced AVD.